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Typhoid toxin disassembly assay

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An abbreviated version of this protocol was published in PLoS Pathogens in Apr 2019

Unique features in the intracellular transport of typhoid toxin revealed by a genome-wide screen

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Detailed protocol

Abstract

Typhoid toxin encoded by typhoidal Salmonella plays a critical role in the pathogenesis of typhoid fever by intoxicating a variety of cell types. This toxin belongs to the AB toxin family and possesses two A subunits CdtB and PltA, and a homopentameric complex comprised of the B subunit PltB. The B subunit (PltB) mediates the cellular entry of typhoid toxin through the retrograde transport pathway. This protocol described the disassembly assay of typhoid toxin occurred in the lumen of the ER during the toxin entry.

Materials and Reagents

-DPBS
-Purified typhoid toxin
-Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Gibco®) containing 10% FBS
-Lysis buffer containing 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 0.5% Triton-100, 1X protease inhibitor cocktail (Roche)
-Nickel resin (Qiagen)
-DTT
-Elution buffer containing 200 mM imidazole and 0.15 M Tris-HCl (pH 6.8)

Equipment

10 cm cell culture dishes
Centrifuge
Microcentrifuge
Cell scraper
Laminar flow hood
Pipetteman

Procedure

Day 1: HEK293T cells ($1x10^7$) are seeded on 10 cm dishes.
Day 2:

1. HEK293T cells are treated with 100 ng of purified His-tagged typhoid toxin at 37 ˚C for 30 minutes.
2. Wash the cells with DPBS once and then incubate in media containing 10% FBS.
3. Collect cells at indicated times and lyse in 1 ml lysis buffer for 30 min at 4 ˚C.
4. Centrifuge the eppendorf at 14,000 rpm for 15 min at 4 ˚C.
5. Transfer the supernatant to a clean eppendorf.
6. Add 20 µL nickel resin and incubate at 4 ˚C overnight.

Day 3:

1. Elute the toxin complex with elution buffer for 20 min at room temperature.
2. Centrifuge the eppendorf at 1,000 rpm for 1 min to pellet the nickel resin.
3. Collect the supernatant and analyze by western blot.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. CHANG, S. (2021). Typhoid toxin disassembly assay. Bio-protocol Preprint. bio-protocol.org/prep1252.
2. Chang, S., Jin, S. C., Jiao, X. and Galán, J. E.(2019). Unique features in the intracellular transport of typhoid toxin revealed by a genome-wide screen. PLoS Pathogens 15(4). DOI: 10.1371/journal.ppat.1007704